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Short running title: Variability in urinary metal levels

ABSTRACT

Background: Metals in single spot urine samples are often used to estimate individual exposure in human studies. However, measurements in urine could vary greatly over time due to the variable exposure, potentially leading to exposure misclassification.

Objective: To examine the variability of metal levels in the urine of 11 men who provided 529 samples on 8 days during a 3-month period, which corresponds to the duration of spermatogenesis.

Method: The urinary levels of arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), molybdenum (Mo) and nickel (Ni) were measured using inductively coupled plasma-mass spectrometry. We calculated the intraclass correlation coefficients (ICCs) to assess the reproducibility of metal measures and computed the sensitivity and specificity to evaluate how well spot urine samples determined the individuals' 3-month average exposure.

Results: Fair to good reproducibility was observed for the serial measurements of Cd (ICC=0.53) in spot samples collected during the 3-month period, whereas the serial measurements of As, Co, Cu, Pb, Mo and Ni showed poor reproducibility (ICCs=0.01-0.29). Use of single spot urine samples to classify the high (top 33%) 3-month average metal levels had uniformly high specificities (0.70-0.84) but relatively low sensitivities (0.40-0.57), except for Cd (0.77). The minimum number of specimens (k) required to estimate the participant-specific mean for the seven metals within 20% of the "true" values ranged from 3 for Cd to 27 for Ni.

Conclusions: The high variability observed in the urinary levels of As, Co, Cu, Pb, Mo and Ni

suggest that a single measurement provides only a brief snapshot in time of the exposure levels

of an individual, which can result in a moderate degree of exposure misclassification.

INTRODUCTION

Metals, including essential and nonessential elements, are naturally present in the environment at

highly varying concentrations and are also widely used in industrial processes and human

activities; humans can be exposed to metals either voluntarily through supplementation or

involuntarily through intake of contaminated food and water and inhalation of ambient air

pollution (Meeker et al. 2010). Consequently, detectable levels of metals have been reported in

most human biological samples from numerous studies worldwide including in China (Canada

2010; CDC 2009; Feng et al. 2014; Kile et al. 2009; Smolders et al. 2014). Toxic elements such

as arsenic (As), cadmium (Cd) and lead (Pb) have been associated with impaired human semen

quality and sperm DNA integrity at relatively low levels (Meeker et al. 2008; Telisman et al.

2007). Essential elements, such as cobalt (Co), copper (Cu), molybdenum (Mo) and nickel (Ni),

are necessary for good health but have also been reported to be associated with changes to male

reproductive health measures, such as reduced semen quality and altered serum levels of

testosterone if above specific levels in animals and in humans (Danadevi et al. 2003; Lewis and

Meeker 2015; Meeker et al. 2008; Pedigo et al. 1988).

Measurements of metals in urine are frequently used as biomarkers in human investigations,

especially in surveys involving a large number of participants, such as the National Health and

Nutrition Examination Survey (NHANES) in the USA (CDC 2014) and the Canadian Health

Measures Survey (CHMS) in Canada (Health Canada 2010). A spot urine sample is the most

commonly used sample type because its collection is relatively simple and non-invasive.

However, metals in spot samples are likely to vary over time due to the variable exposure driven

by changes in diet, lifestyle or the daily activities of a person (Barr et al. 2005; Smolders et al.

2014). That variation may be more pronounced for metals that are rapidly eliminated after

external exposure. Correction for urinary dilution, such as creatinine-corrected concentrations,

may introduce an additional source of variability that does not reflect urine dilution (Barr et al.

2005). Because most reproductive health end points of interest, such as sperm quality, are likely

associated with metal exposures over time intervals of months, concerns have been raised as to

whether single measurements of metals in spot urine samples accurately reflect individual

exposure over time (Smolders et al. 2014).

Several previous studies have examined the temporal variability in the urinary levels of As, Cd

and Ni. In a Bangladesh study, Kile et al. (2009) found fair to good reproducibility in urinary As

concentrations but poor reproducibility in percent As species among 196 participants who

provided repeated spot urine samples during a 2-year period. In a US study conducted with 141

women, Gunier et al. (2013) revealed that Cd concentrations in repeated 24-h urine samples were

only fair to good reproducible over 3-9 months. A more recent study in Belgium collecting spot

urine samples from 8 adults also reported poor reproducibility in urinary As and Ni

concentrations over a 6-day period (Smolders et al. 2014). Differences in the source and extend

of exposure (e.g., smoking status, water consumption, fish intake) may contribute to some of the

differences among these studies. However, the variability patterns of metals in spot, first

morning and 24-h urine samples obtained from the same person during a 3-month period, which

corresponds to the duration of spermatogenesis, remain unclear.

To address the data gap, we conducted this study in 11 men with the intention to (1) assess the

reproducibility of serial measurements of As, Cd, Co, Cu, Mo, Ni and Pb in urine during a

3-month period; (2) assess the effect of concentration corrections, including creatinine

correction, creatinine as a covariate and the urinary excretion rate (UER) calculation, on the

reproducibility of metal measurements; (3) evaluate the correlation between spot, first morning

and 24-h urine samples collected within 24 h; and (4) evaluate the sensitivity and specificity of

spot samples that were used to classify the individuals' 3-month average excretion

measurements.

MATERIALS AND METHODS

Study population and sample collection

The research protocol was approved by the Ethics Committee of Tongji Medical College. From

September 2012 to February 2013, 11 men were recruited to participate in a study designed to

examine the variability in urinary levels of dichloroacetic acid and trichloroacetic acid (Wang et

al. 2014). The study volunteers were non-smoking healthy men living in a restricted

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geographical area in Wuhan, China, aged 21 to 28 years (mean age: 23.64±1.96 years), with no

history of diabetes or renal disease and no documented occupational exposure to metals. Each

participant gave written informed consent before participation.

Basic study details have been previously described (Wang et al. 2014). In brief, during the study

period over 3 months, 11 participants were asked to provide urine samples on days 0, 1, 2, 3, 4,

30, 60, and 90. The participants collected all urine samples in a trace-elements free polyethylene

specimen container on the sampling days. After recording the total volume and time of each

void, the urine was decanted in a prelabeled, trace-elements free polyethylene urine cup and

stored at -40 °C until analysis (within 6 months). A total of 529 out of 535 possible spot urine

samples were collected during the study period (six urine voids were not collected), including 88

first morning urine samples (one for each study participant on each of the 8 sampling days). The

participants had no food or drinks restrictions, but they were asked to complete a food diary that

recorded detailed information on food consumption during the sampling period.

Metal determination

The concentrations of As, Cd, Co, Cu, Mo, Ni and Pb in the urine were analyzed using a method

described in detail in our recent study (Feng et al. 2014). Briefly, a 3.0-mL aliquot of urine was

transferred to a polyethylene tube containing 15 μL of 67% (v/v) HNO_3 (Optima[TM] grade,

Fisher) and stored in a refrigerator at 5 °C for at least 24 hours. After the urine samples were

brought to room temperature, 1.0 mL of the sample was diluted to 5.0 mL with 1.2% (v/v) HNO_3

(Optima[TM] grade, Fisher), which was then detected using an Agilent 7700x inductively coupled

plasma mass spectrometer with an octopole-based collision/reaction cell (Agilent Technologies).

We used the standard reference material (SRM) 2670a and 1640a (both purchased from National

Institute of Standards and Technology), as well as a spiked pooled sample of 100 urines

randomly selected from samples collected for the present study as a quality control. SRM 2670a

and spiked urine were analyzed at low and high concentrations for each element after a

calibration procedure to evaluate the method's accuracy. SRM 1640a was measured every 20

samples to ensure instrument performance. The spike recoveries for the seven metals ranged

from 85% to 114% and the coefficient of variance (intra-day and inter-day variation) was not

higher than 10.00%. Additionally, each batch included one blank urine sampling tube containing

3-mL deionised water, which was analyzed using the same protocols as described for the urine

samples. Analyte concentrations were below the limits of quantification (LOQ) in all blank

samples. The LOQs for As, Cd, Co, Cu, Mo, Ni and Pb were 0.013 µg/L, 0.002 µg/L, 0.001

µg/L, 0.234 µg/L, 0.004 µg/L, 0.035 µg/L and 0.178 µg/L, respectively. Values below the LOQ

were assigned to LOQ/$\sqrt{2}$ for analysis.

Urinary metal concentrations/excretions were expressed in the following three ways: (1) as an

uncorrected concentration (µg/L); (2) as a creatinine-corrected concentration, calculated by

dividing the uncorrected concentration by the urinary creatinine concentration (µg/g creatinine);

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and (3) as the urinary excretion rate (UER) of each metal, computed by dividing the total mass of excretion by the amount of time since the previous void (μg/h) (Akerstrom et al. 2013). We also adjusted for urine dilution by including creatinine as a model covariate (Kim et al. 2011). Creatinine concentrations were measured using a commercial test kit based on the picric acid assay (Jiancheng Bioengineering Ltd.). We defined spot samples as all individual urine voids collected during a given day, including the first morning samples. We defined first morning urine samples as the first sample collected from an individual at or after 5:00 AM each day and 24-h urine collections as the volume-weighted average of all specimens collected from an individual during a 24-h period starting at midnight (Wang et al. 2014).

Data analysis

Data analyses were performed using Statistical Analysis Software (SAS), version 9.2 (SAS Institute, Cary, NC). We first conducted descriptive statistics and assessed normality. Because of the skewed distribution of the urinary metal measurements, $\log_{10}$-transformed values were used for subsequent analyses.

We constructed a line chart to visually compare the within-person and between-person variability of urinary metal levels across the 3-month period. To quantitatively assess the variance component of the metals, we further estimated the within-person and between-person variance for each sample type (spot, first morning and 24-h urine samples) based on output from

multilevel random-effects models. We estimated the between-person, within-person

between-day, and within-person within-day variances for spot samples; whereas, for first

morning and 24-h urine samples, we calculated only the between-person and within-person

variances because one value was available each day. We report the variance apportionments of

metals separately for samples collected days apart only, for samples collected months apart only,

and for all urine samples. The variances for "days apart" samples are based on urine samples

collected on days 0, 1, 2, 3, and 4 (5 sampling days total) and variances for "months apart"

samples are based on urine samples collected on days 0, 30, 60, and 90 (4 sampling days total).

Akaike information criterion (AIC) values (lower AIC values are associated with better models)

were calculated to assess the fit of the models (uncorrected concentrations, creatinine-corrected

concentrations, creatinine as a covariate and UERs) based on all spot samples (n=529). ICCs

were computed to assess the reproducibility of repeated measurements. The ICC, ranging from 0

(low reproducibility) to 1 (high reproducibility), is the ratio of the between-person variance to

the sum of the between-person and within-person variance. We used standardized criteria

proposed by Rosner (2002) to evaluate the ICCs: poor reproducibility (ICC<0.40), fair to good

reproducibility (0.40≤ICC<0.75) and excellent reproducibility (ICC≥0.75). We also estimated

the variance apportionment of creatinine concentrations in the three sample types.

We used Pearson's correlation analyses to examine the correlation between the metal

concentrations measured in a spot (randomly selected each day) or first morning urine sample

and the concentrations measured in the same-day 24-h collection. We also constructed mixed

regression models to assess the efficacy of metal concentrations in same-day spot (randomly

selected each day) or first morning urine samples as predictors of their 24-h urine levels. The

coefficient of determination (R^2) was estimated to assess the predictive power of the model.

We finally evaluated how well one, two, or three randomly selected spot urine samples collected

on different days could correctly classify the participants into the high (top 33%) exposure group

based on their 3-month average measurements by comparing the distribution of "true" and

"predicted" levels for agreement according to the method described by Bradman et al. (2013).

For the "true" exposure, we calculated the arithmetic mean of metal concentrations ($\log_{10}$ scale)

for each person using all their spot samples (including first morning samples) collected during

the 3-month period, categorized the values into tertiles, and identified the "true" top 33%

exposure levels. For the "predicted" levels, we created 10 data sets each containing one randomly

selected urine sample per person. Within each randomly-selected data set, we categorized the 11

randomly selected concentrations into tertiles, and also identified the "predicted" top 33%

exposure levels. We reported the average sensitivity and specificity observed across the 10

separate random samples. To determine whether collection of repeated urine samples improved

the accuracy of classification, we repeated the analysis using the arithmetic mean of the

$\log_{10}$-transformed metal concentrations in two or three urine samples randomly selected from

each participant collected on different days. The sensitivity and specificity were separately

calculated for all spot samples and for first morning urine samples only. We further calculated

the minimum number of spot samples (including first morning samples) (*k*) needed to estimate

the participant-specific mean for metals within 20% of the ''true'' values with a probability of

95% after a $\log_{10}$ transformation without regard to the day of sample collection, using the

equation: $k=(1.96 \times CV/20)^2$, where CV is the within-person coefficient of variation (Kim et al.

2014).

RESULTS

The basic characteristics of the 11 participants are presented in Supplemental Material, Table S1.

Three participants reported eating fish on one occasion during the three-month sampling period,

the other participants did not eat any fish. None of the participants ate seafood other than fish

during the sampling period. The participants urinated between 3 and 11 times per day (mean:

6.01 voids), and the volume of voids ranged from 20.3 to 740.6 mL (mean: 195.4 mL). The

distribution of metal concentrations in spot, first morning and 24-h urine samples are presented

in Supplemental Material, Table S2. All seven metals were detected in greater than 95% of the

529 spot samples.

Figure 1 presents the creatinine-corrected concentrations of metals (µg/g creatinine) in spot urine

samples collected from the 11 men on 8 days during the 3-month period. There were no

pronounced cyclic patterns in the metal concentrations among the 11 participants, although the

line charts showed that some metal concentrations in urine varied up to two (e.g., As, Co, Mo) or

three (e.g., Cu, Ni, Pb) orders of magnitude during the period. The concentrations of Cd in spot

samples were rather consistent and varied within an order of magnitude for most participants

(P2, P3, P4, P5, P6, P8, P9, P10, P11; Figure 1).

We used four different models to evaluate the influence of concentration corrections on the

reproducibility of metal measurements in spot samples (Table 1). The highest AIC values for the

multilevel random-effects models based on all spot urine samples were achieved with the

uncorrected values for most metals, which indicated the worst fit of the models. The AIC

decreased if we accounted for urinary dilution. The apportionments of the within-person and

between-person variances for metal measurements were similar based on the

creatinine-corrected, creatinine as a covariate and UER models. Specifically, we found that

accounting for urinary dilution increased the between-person relative to the within-person

variance for Cd and Co but decreased the variance for As and Cu. The best model fit was

achieved with the creatinine-corrected model for Co, Mo and Ni, and the creatinine as a

covariate model for As, Cd, Cu and Pb. Because the variance apportionments of metals estimated

from both models were similar and the 24-h voids data cannot be accounted for urine dilution by

modeling creatinine as a covariate, we used only the creatinine-corrected values in subsequent

analyses.

The within-person and between-person variance apportionments of creatinine-corrected metal

concentrations in spot, first morning and 24-h urine samples collected from 11 adults on 8 days

during the 3-month period are shown in Table 2. Fair to good reproducibility was observed for

serial measurements of Cd (ICC=0.53) in spot samples during the 3-month period, whereas serial

measurements of As, Co, Cu, Pb, Mo and Ni showed poor reproducibility (ICCs=0.01-0.29). For

As, Co, Cu, Pb, Mo and Ni, the total within-person variance (between-day + within-day)

(71-99%) was much higher than the between-person variance (1-29%), and the within-day

(42-82%) variance was the largest component of the total variance. Compared to spot urine

samples, the serial measurements of Cd (ICC=0.68 and 0.70 for first morning and 24-h urine

samples, respectively) and Co (ICC=0.55 and 0.50 for first morning and 24-h urine samples,

respectively) in first morning and 24-h urine samples provided apparently higher consistency;

however, the advantages of these two sample types over spot samples were not as evident for the

other metals. We re-analyzed the data by including "time since the previous urination" as a

model covariate and found that the estimated variance apportionments for the metals in all spot

samples remained largely unchanged (percentage differences in estimated variance

apportionments between models ranged from 0.0% to 37.5%).

We also separately calculated the variance apportionments of creatinine-corrected metal

concentrations in the three sample types based on samples collected days apart (including

samples collected on days 0, 1, 2, 3, and 4; total *n*=326) and months apart (including samples

collected on days 0, 30, 60, and 90; total *n*=265) (Table 3). If urine samples were collected days

apart, fair to good reproducibility were obtained for As (ICC=0.41), Cd (ICC=0.64) and Co

(ICC=0.41) in spot samples; however, poor reproducibility (ICCs=0.01-0.18) were observed for

the other metals. Compared to spot urine samples, higher ICCs were observed for Cd (0.77), Co

(0.74), Cu (0.44) and Pb (0.36) in first morning urine samples, as well as Cd (0.82) and Co (0.69)

in 24-h collections. For urine samples collected months apart, decreased ICCs were observed for

the seven metals in each sample type, and fair to good reproducibility were obtained for only Cd

(ICCs=0.51-0.65). The reproducibility of creatinine in spot samples within individuals were poor

over periods ranging from days to months (ICCs=0.09-0.15), but were fair to good in first

morning and 24-h urine samples (ICCs=0.49-0.62) (see Supplemental Material, Table S3).

Scatter plots of correlations between creatinine-corrected metal concentrations in the three

sample types collected within the same-day 24-h period are presented in Figure 2. The

creatinine-corrected metal concentrations in a spot or first morning urine sample were

significantly correlated with those measured in the same-day 24-h urine collection (all *P*-value

<0.001). The abilities of same-day spot or first morning urine samples to predict 24-h urine metal

excretions based on mixed regression models are presented in Table 4. For models examining a

spot sample and its respective 24-h sample, the predictive power of the models were high for Cd

(R^2=0.76) and Co (R^2=0.71) but only low-to-moderate for the other metals (R^2=0.29-0.69).

Apparent advantage of using a first morning urine sample rather than a spot sample to predict the

respective 24-h collection was observed for only As (R^2= 0.81 for first morning samples vs. 0.67

for spot samples).

The results from sensitivity and specificity analysis of the ability to correctly classify the

participants into a high exposure group (based on 3-month average concentrations) based on one,

two, or three randomly selected urine samples collected on different days are presented in Table

5. The proportion of participants who truly had the highest 3-month average exposure (top 33%)

that would be correctly classified as such using a single urine sample (the sensitivities) were high

for Cd (0.77 for spot samples and 0.83 for first morning samples) but relatively low for the other

metals (range: 0.37-0.57). Use of two spot samples collected days apart provided ≤10% increase

in sensitivities for most metals. If three spot urine samples were collected days apart,

moderate-to-high sensitivities in the range of 0.63-0.97 were obtained for As, Cd, Co, Cu, Pb and

Mo. Within the first morning sample group, using two samples collected days apart to classify

the individual's exposure provided ≥15% increase in sensitivities over a single sample for As,

Co, Cu and Ni; if three samples were collected days apart, moderate-to-high sensitivities in the

range of 0.60-0.97 were observed for As, Cd, Co, Cu, Mo and Ni. The specificity was fair to

good and uniformly higher than the sensitivity in each type of sample collection. The collection

of urine samples months apart did not offer an advantage in exposure classification over samples

collected days apart. The minimum number of specimens (k) required to estimate the

participant-specific mean for the seven metals within 20% of the ''true'' values ranging from 3

for Cd to 27 for Ni (Table 5).

DISCUSSION

The present study found substantial within-person variation in the serial measurements of metals

in urine samples in 11 men during a 3-month period. We obtained fair to good reproducibility for

the creatinine-corrected As, Cd and Co concentrations in spot samples collected on 5 consecutive

days (ICCs=0.41-0.64), suggesting that urinary As, Cd and Co concentrations were fairly stable

over the short term. For Cu, Pb, Mo and Ni, however, relatively low ICCs in the range of

0.01-0.18 were observed. The ICCs of the metals decreased if urine samples were collected

further apart in time, and fair to good reproducibility were observed for only Cd (ICC=0.51). The

decline in ICCs is not unexpected because the collection of samples months apart includes

variability in urinary metal concentrations that contributed to both day-to-day changes and

monthly trends in metal exposure, such as seasonal variation in diet, lifestyle, and activity

patterns, as well as other environmental or biologic factors (Hauser et al. 2004). However, in this

study, the collection of urine samples months apart did not offer an advantage in exposure

classification over samples collected days apart, which suggests that urine sample collected days

apart may be as good as samples collected months apart.

Twenty-four-hour urine samples have been recommended by some as the "gold standard" for evaluating personal exposure to toxic chemicals that are excreted mainly through the urine (Bradman et al. 2013; EPA 1996). In this study, although metal concentrations in spot urine samples were significantly correlated with those in the same-day 24-h urine samples, only low-to-moderate predictive power was achieved for As, Cu, Pb, Mo and Ni (R^2=0.29-0.69) using creatinine-corrected spot urine samples as predictors. This finding implies that measurements of these metals in a single spot urine sample may not be good surrogates for those in 24-h collection. Many studies have instead used first morning urine samples to evaluate personal exposure because they are more concentrated and correlated with the 24-h urine collection (Kissel et al. 2005; Scher et al. 2007). However, in the present study, after correction for urine dilution, the levels of metal in spot and first morning samples were similar and the advantage of using a first morning urine sample rather than a spot sample to predict the respective 24-h collection was evident for only As. Moreover, compared with spot samples, the use of first morning and 24-h urine collections only improved the consistency of serial measurements for Cd and Co. The high between-day (within-person) variance of As, Cu, Pb, Mo, and Ni in first morning and 24-h urine samples (71%-99%) suggests that concentrations in a single first morning or 24-h urine sample may not provide accurate estimates of individual exposures over weeks or months.

Our findings support the use of creatinine-corrected metal concentrations to control for urinary

dilution among adult men. Our findings suggested that the differences in urine dilution most

likely explained some of the observed variation in concentrations of most metals because the

model fits improved if we accounted for urine dilution. Consistent with this notion, we found a

rather poor reproducibility of urinary creatinine concentrations in spot samples within

individuals. The UER calculation, which is a measure of the chemical mass in the sample, has

been proposed as an appropriate method to eliminate the variability in urine concentrations from

urine dilution (Barr et al. 2005). The similar apportionment of the within-person and

between-person variance for metals based on creatinine-corrected and UER models suggests that

other sources of variability introduced by creatinine correction (e.g., age, body size, muscle

mass) may not be major contributing factors to the observed variation, which reflects true

variations in metal excretions. Alternatively, when we included creatinine as a model covariate

instead of creatinine-corrected values to account for urine dilution, the variance apportionments

of metals remained largely unchanged. The apportionments of variance were also robust to the

adjustment for "time since the previous urination" (data not shown), which was proposed as an

important parameter to take into account when considering the variability of urinary metal

measures (Smolders et al. 2014). However, we did not quantify specific gravity in these samples.

Additional research is needed to confirm whether our findings are valid for adult men using

specific gravity to account for urinary dilution.

The reproducibility of serial measurements of metal in urine may be affected by the kinetics of absorption, distribution, metabolism and elimination of the analyte, particularly the relationship between elimination half-life and the intervals between exposure events (Aylward et al. 2014). The possible reason that urinary Cd concentrations were fairly stable during a 3-month period could be related to frequent exposure and Cd's long biological elimination half-life. Cd is stored in the liver and kidneys with a half-life of 10-30 years and therefore can easily reach a steady state from chronic exposure via ingestion (Gunier et al. 2013). Fair-to-excellent reproducibility of urinary levels of Cd has been revealed in two previous studies of 296 women who collected repeated 24-h urine samples over 3-9 months (creatinine-corrected ICC=0.42) (Gunier et al. 2013) and of 8 adults who provided spot urine samples during a 6-day period (creatinine-corrected ICC=0.75) (Smolders et al. 2014). Serial measurements of other metals were more variable than those of Cd throughout the day and across days most likely due to their much shorter elimination half-life (Aylward et al. 2014). In support of our findings, studies by Smolders et al. (2014) also reported poor reproducibility for the urine levels of As (creatinine-corrected ICC=0.21). However, our findings differed from those of the studies by Navas-Acien et al. (2009) that found urinary As concentrations were fairly stable during 10 years in 60 U.S. adults with stable environmental exposure to inorganic arsenic in drinking water; and Smolders et al. (2014) that observed fair to good reproducibility for serial measurements of Ni (creatinine-corrected ICC=0.68) among four couples who consumed fish frequently. The varied

ICCs between studies may be due in part to the differences in duration between repeat urine

sample collections (Smolders et al. 2014). Study design, source and extend of exposure, gender,

age, the underlying physiology of the participants and geography where interaction with sources

of exposure may differ can also partly explain the variation (Smolders et al. 2014).

In our sensitivity and specificity analysis, we observed similar trends to those of the ICCs. For

example, use of single spot urine samples to classify the high (top 33%) 3-month average metal

levels had high sensitivity for Cd (0.77) but relatively low sensitivities for the other metals

(0.40-0.57). This finding implies that in relying on a single urine sample to categorize an

individual's average exposure levels of As, Co, Cu, Mo, Ni and Pb over periods of days or

months, there is likely exposure misclassification that tends to attenuate risk estimations. In our

study population, three specimens collected at different times were necessary to estimate

participant-specific means within 20% of the ''true'' values for Cd; whereas more than 27

specimens were required for Ni, which is likely a consequence of poorer reliability secondary to

lower concentrations (Kim et al. 2014). However, it should be noted that the calculated

sensitivities and specificities may be slightly overestimated because we included "predicted"

values in the calculation of the "true" values. A portion of the increased sensitivities and

specificities observed when selecting two or three samples for each subject instead of a single

sample may also be caused partly by the increased dependence between the errors of the

"predicted" and "true" values (Hauser et al. 2004).

The strengths of the present study include its large sample size per subject and serial measurements of metals in three types of urine samples over durations relevant to male reproductive health. The median urinary levels of metals in this study are similar to those reported in previous studies among Chinese adults (Feng et al. 2014). However, our median urinary levels of As (19.86 μg/L) and Pb (2.75 μg/L) were much higher than those reported for the males from NHANES in the USA (CDC 2014) (8.80 μg/L for As; 0.54 μg/L for Pb) and CHMS in Canada (Health Canada 2010) (12.62 μg/L for As; 0.60 μg/L for Pb), indicating higher As and Pb exposure in our population that may reduce the contribution of the within-person variance to the total variance. In addition, it is likely that between-person variability in our study population of healthy non-smoking young adult men, who did not use dietary supplements and only rarely consumed fish, was lower than in the general population. Moreover, our findings may not be generalizable to other study populations, such as children or pregnant women, because differences in exposure patterns (e.g., related to diet and lifestyle), and possibly age- and gender-related differences in metabolism, will contribute to variation (Aylward et al. 2014; Smolders et al. 2014). Finally, we did not analyze duplicate aliquots to assess variability due to analytic factors, which may bias ICCs as an estimate of between-person variability within our study population.

CONCLUSIONS

In our study population of adult nonsmoking Chinese men, serial measures of Cd in spot urine

samples had fair to good reproducibility over a 3-month period. However, the serial measures of

As, Co, Cu, Pb, Mo and Ni showed poor reproducibility, and the use of single spot samples to

classify the individuals' 3-month average exposure can result in a moderate degree of exposure

misclassification. Between-day variance of As, Cu, Pb, Mo, and Ni based on a single first

morning void or a single 24-h urine sample was similar to between-day variance based on a

single spot sample, which suggests that improvements in long-term exposure estimation may not

offset the additional effort required to collect first morning or 24-h samples. Collection of

multiple urine samples from each study participant is an option for reducing exposure

classification errors, and it is valuable to consider the number of specimens required to estimate

participant-specific mean values to maximize study efficiency. However, our study does not

allow concluding that urine samples collected further apart in time offer an advantage in

exposure classification.

REFERENCES

Akerstrom M, Sallsten G, Lundh T, Barregard L. 2013. Associations between urinary excretion

of cadmium and proteins in a nonsmoking population: Renal toxicity or normal physiology?

Environ Health Perspect 121:187-191.

Aylward LL, Hays SM, Smolders R, Koch HM, Cocker J, Jones K, et al. 2014. Sources of

variability in biomarker concentrations. J Toxicol Env Heal B 17:45-61.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. 2005. Urinary creatinine

concentrations in the u.S. Population: Implications for urinary biologic monitoring

measurements. Environ Health Perspect 113:192-200.

Bradman A, Kogut K, Eisen EA, Jewell NP, Quiros-Alcala L, Castorina R, et al. 2013.

Variability of organophosphorous pesticide metabolite levels in spot and 24-hr urine samples

collected from young children during 1 week. Environ Health Perspect 121:118-124.

Health Canada. 2010. Report on Human Biomonitoring of Environmental Chemicals in Canada.

Results of the Canadian Health Measures Survey Cycle 1 (2007-2009). Available at:

http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/chms-ecms/re

port-rapport-eng.pdf [accessed 10 September 2014].

CDC (Centers for Disease Control and Prevention). 2014. Fourth national report on human

exposure to environmental chemicals. Updated Tables, August, 2014. Available at:

http://www.cdc.gov/exposurereport/pdf/fourthreport_updatedtables_aug2014.pdf [accessed 10

September 2014].

Danadevi K, Rozati R, Reddy PP, Grover P. 2003. Semen quality of indian welders

occupationally exposed to nickel and chromium. Reprod Toxicol 17:451-456.

EPA (Environmental Protection Agency). 1996. Occupational and residential exposure test

guidelines. Oppts 875.1500. Biological monitoring. Available at:

http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series875.htm [accessed 10

September 2014].

Feng W, He X, Chen M, Deng S, Qiu G, Li X, et al. 2015. Urinary metals and heart rate

variability: A cross-sectional study of urban adults in wuhan, China. Environ Health Perspect

123:217-222.

Gunier RB, Horn-Ross PL, Canchola AJ, Duffy CN, Reynolds P, Hertz A, et al. 2013.

Determinants and within-person variability of urinary cadmium concentrations among women

in northern california. Environ Health Perspect 121:643-649.

Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. 2004. Temporal variability of urinary

phthalate metabolite levels in men of reproductive age. Environ Health Perspect

112:1734-1740.

Kile ML, Hoffman E, Hsueh YM, Afroz S, Quamruzzaman Q, Rahman M, et al. 2009.

Variability in biomarkers of arsenic exposure and metabolism in adults over time. Environ

Health Perspect 117:455-460.

Kim K, Steuerwald AJ, Parsons PJ, Fujimoto VY, Browne RW, Bloom MS. 2011.

Biomonitoring for exposure to multiple trace elements via analysis of urine from participants

in the study of metals and assisted reproductive technologies (SMART). J Environ Monit

13:2413-2419.

Kim K, Bloom MS, Fujimoto VY, Browne RW. 2014. Number of specimens required to

estimate average follicular fluid high-density lipoprotein particle components in women

undergoing in vitro fertilization. Fertil Steril 101:e44.

Kissel JC, Curl CL, Kedan G, Lu C, Griffith W, Barr DB, et al. 2005. Comparison of

organophosphorus pesticide metabolite levels in single and multiple daily urine samples

collected from preschool children in washington state. J Expo Anal Environ Epidemiol

15:164-171.

Lewis RC, Meeker JD. 2015. Biomarkers of exposure to molybdenum and other metals in relation to testosterone among men from the united states national health and nutrition examination survey 2011-2012. Fertil Steril 103:172-178.

Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, et al. 2008. Cadmium, lead, and other metals in relation to semen quality: Human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect 116:1473-1479.

Meeker JD, Rossano MG, Protas B, Padmanahban V, Diamond MP, Puscheck E, et al. 2010. Environmental exposure to metals and male reproductive hormones: Circulating testosterone is inversely associated with blood molybdenum. Fertil Steril 93:130-140.

Navas-Acien A, Umans JG, Howard BV, Goessler W, Francesconi KA, Crainiceanu CM, et al. 2009. Urine arsenic concentrations and species excretion patterns in american indian communities over a 10-year period: The strong heart study. Environ Health Perspect 117:1428-1433.

Pedigo NG, George WJ, Anderson MB. 1988. Effects of acute and chronic exposure to cobalt on male reproduction in mice. Reprod Toxicol 2:45-53.

Rosner B. 2002. Multisample inference. In: Rosner B (ed) Fundamentals of biostatistics, 5th edn Pacific Grove, Duxbury, pp 511-576.

Scher DP, Alexander BH, Adgate JL, Eberly LE, Mandel JS, Acquavella JF, et al. 2007.

Agreement of pesticide biomarkers between morning void and 24-h urine samples from

farmers and their children. J Expo Sci Environ Epidemiol 17:350-357.

Smolders R, Koch HM, Moos RK, Cocker J, Jones K, Warren N, et al. 2014. Inter- and

intra-individual variation in urinary biomarker concentrations over a 6-day sampling period.

Part 1: Metals. Toxicol Lett 231:249-260.

Telisman S, Colak B, Pizent A, Jurasovic J, Cvitkovic P. 2007. Reproductive toxicity of

low-level lead exposure in men. Environ Res 105:256-266

Wang YX, Zeng Q, Wang L, Huang YH, Lu ZW, Wang P, et al. 2014. Temporal variability in

urinary levels of drinking water disinfection byproducts dichloroacetic acid and trichloroacetic

acid among men. Environ Res 135C:126-132.

Table 1. The variance apportionment of log_{10}-transformed metal concentrations/excretions in spot samples collected from 11 adults (*n*=529).

	As	Cd	Co	Cu	Pb	Mo	Ni
Uncorrected (µg/L)							
AIC[a]	700.7	496.9	404.4	402.8	401.3	730.1	1106.0
Between persons σ^2 (%)[b]	0.052 (67%)	0.048 (25%)	0.043 (25%)	0.033 (21%)	0.008 (6%)	0.011 (4%)	0.001 (1%)
Within person, between day σ^2 (%)[c]	0.025 (32%)	0.020 (10%)	0.025 (15%)	0.015 (13%)	0.031 (22%)	0.039 (16%)	0.175 (31%)
Within person, within day σ^2 (%)[d]	0.001 (1%)	0.126 (65%)	0.102 (60%)	0.107 (69%)	0.102 (72%)	0.198 (80%)	0.376 (68%)
Creatinine-corrected (µg/g creatinine)							
AIC[a]	204.6	278.1	16.6	314.0	570.1	239.7	965.8
Between persons σ^2 (%)[b]	0.012 (19%)	0.046 (53%)	0.027 (29%)	0.010 (8%)	0.009 (5%)	0.002 (2%)	0.001 (1%)
Within person, between day σ^2 (%)[c]	0.026 (39%)	0.016 (18%)	0.022 (24%)	0.020 (17%)	0.023 (13%)	0.038 (34%)	0.163 (36%)
Within person, within day σ^2 (%)[d]	0.028 (42%)	0.025 (29%)	0.043 (47%)	0.088 (75%)	0.149 (82%)	0.071 (64%)	0.281 (63%)
Creatinine as a covariate							
AIC[a]	31.3	64.8	34.5	204.0	359.3	369.2	980.4
Between persons σ^2 (%)[b]	0.009 (11%)	0.040 (42%)	0.030 (32%)	0.013 (13%)	0.005 (4%)	0.001 (1%)	0.001 (1%)
Within person, between day σ^2 (%)[c]	0.024 (30%)	0.017 (18%)	0.021 (23%)	0.017 (17%)	0.027 (21%)	0.037 (28%)	0.156 (35%)
Within person, within day σ^2 (%)[d]	0.047 (59%)	0.039 (40%)	0.041 (55%)	0.070 (70%)	0.094 (75%)	0.094 (71%)	0.289 (64%)
Urinary excretion rate (µg/h)							
AIC[a]	248.6	223.9	351.7	547.6	705.4	495.7	1027.5
Between persons σ^2 (%)[b]	0.019 (16%)	0.051 (38%)	0.045 (29%)	0.012 (7%)	0.006 (3%)	0.008 (5%)	0.001 (1%)
Within person, between day σ^2 (%)[c]	0.023 (20%)	0.011 (8%)	0.013 (8%)	0.010 (6%)	0.017 (8%)	0.031 (19%)	0.159 (33%)
Within person, within day σ^2 (%)[d]	0.075 (64%)	0.074 (54%)	0.096 (63%)	0.149 (87%)	0.201 (89%)	0.124 (76%)	0.322 (66%)

σ^2= variance. [a]Akaike information criterion (AIC) values is used to compare the fit of models. [b]The proportion of between-person variance to the total variance. [c]The proportion of within-person between-day variance to the total variance. [d]The proportion of within-person within-day variance to the total variance.

Table 2. The variance apportionment of $\log_{10}$-transformed creatinine-corrected metal concentrations in the three sample types collected from 11 adults during a 3-month period.

Type of sample	As	Cd	Co	Cu	Pb	Mo	Ni
Spot sample (*n*=529)							
ICC	0.19	0.53	0.29	0.08	0.05	0.02	0.01
Between persons σ^2 (%)[a]	0.012 (19%)	0.046 (53%)	0.027 (29%)	0.010 (8%)	0.009 (5%)	0.002 (2%)	0.001 (1%)
(95% CI)[b]	(0.000, 0.025)	(0.010, 0.082)	(0.004, 0.050)	(0.000, 0.021)	(-0.003, 0.021)	(-0.004, 0.008)	(-0.005, 0.007)
Within person, between day σ^2 (%)[c]	0.026 (39%)	0.016 (18%)	0.022 (24%)	0.020 (17%)	0.023 (13%)	0.038 (34%)	0.163 (36%)
(95% CI)[b]	(0.018, 0.034)	(0.011, 0.021)	(0.014, 0.030)	(0.011, 0.031)	(0.010, 0.036)	(0.025, 0.051)	(0.111, 0.217)
Within person, within day σ^2 (%)[d]	0.028 (42%)	0.025 (29%)	0.043 (47%)	0.088 (75%)	0.149 (82%)	0.071 (64%)	0.281 (63%)
(95% CI)[b]	(0.025, 0.031)	(0.022, 0.028)	(0.038, 0.048)	(0.078, 0.098)	(0.132, 0.165)	(0.063, 0.079)	(0.248, 0.310)
First morning sample (*n*=88)[e]							
ICC	0.28	0.68	0.55	0.20	0.14	0.02	0.01
Between person σ^2 (%)[a]	0.016 (28%)	0.059 (68%)	0.059 (55%)	0.015 (20%)	0.011 (14%)	0.002 (2%)	0.001 (1%)
(95% CI)[b]	(0.001, 0.031)	(0.014, 0.102)	(0.011, 0.107)	(-0.001, 0.032)	(-0.004, 0.026)	(-0.009, 0.013)	(-0.007, 0.011)
Within person σ^2 (%)[c]	0.041 (72%)	0.027 (32%)	0.049 (45%)	0.059 (80%)	0.069 (86%)	0.094 (98%)	0.220 (99%)
(95% CI)[b]	(0.030, 0.052)	(0.020, 0.034)	(0.036, 0.062)	(0.043, 0.075)	(0.051, 0.087)	(0.069, 0.119)	(0.166, 0.274)
24-h collection (*n*=88)[e]							
ICC	0.29	0.70	0.50	0.12	0.07	0.02	0.01
Between person σ^2 (%)[a]	0.013 (29%)	0.048 (70%)	0.034 (50%)	0.009 (12%)	0.006 (7%)	0.001 (2%)	0.001 (1%)
(95% CI)[b]	(-0.112, 0.138)	(0.012, 0.087)	(0.006, 0.062)	(-0.004, 0.022)	(-0.006, 0.018)	(-0.056, 0.059)	(-0.067, 0.070)
Within person σ^2 (%)[c]	0.032 (71%)	0.021 (30%)	0.034 (50%)	0.065 (88%)	0.079 (93%)	0.049 (98%)	0.153 (91%)
(95% CI)[b]	(0.024, 0.040)	(0.016, 0.026)	(0.024, 0.044)	(0.047, 0.083)	(0.058, 0.100)	(0.036, 0.062)	(0.115, 0.191)

σ^2= variance. [a]The proportion of between-person variance to the total variance. [b]Confidence intervals for the variance components. [c]The proportion of within-person between-day variance to the total variance. [d]The proportion of within-person within-day variance to the total variance. [e]For first morning and 24-h urine collections, the distinction between within-day vs. between-day variability is not applicable with only one measurement per day.

Table 3. The variance apportionment of $\log_{10}$-transformed creatinine-corrected metal concentrations in urine samples, collected days apart and months apart, from 11 adults.

Type of sample	As	Cd	Co	Cu	Pb	Mo	Ni
Days apart (*n*=326)							
Spot sample							
ICC	0.41	0.64	0.41	0.18	0.10	0.01	0.12
Between persons σ^2 (%)[a]	0.011 (41%)	0.047 (64%)	0.033 (41%)	0.017 (18%)	0.018 (10%)	0.001 (1%)	0.012 (3%)
Within person, between day σ^2 (%)[b]	0.015 (56%)	0.007 (10%)	0.007 (9%)	0.012 (12%)	0.011 (6%)	0.024 (25%)	0.107 (31%)
Within person, within day σ^2 (%)[c]	0.001 (3%)	0.020 (26%)	0.041 (50%)	0.068 (70%)	0.161 (84%)	0.070 (74%)	0.232 (66%)
First morning sample[d]							
ICC	0.32	0.77	0.74	0.44	0.36	0.01	0.12
Between person σ^2 (%)[a]	0.016 (32%)	0.064 (77%)	0.077 (74%)	0.017 (44%)	0.022 (36%)	0.001 (1%)	0.013 (12%)
Within person σ^2 (%)[b]	0.034 (68%)	0.019 (23%)	0.027 (26%)	0.022 (56%)	0.040 (64%)	0.080 (99%)	0.094 (88%)
24-h collection[d]							
ICC	0.34	0.82	0.69	0.16	0.10	0.06	0.14
Between person σ^2 (%)[a]	0.011 (34%)	0.049 (82%)	0.040 (69%)	0.011(16%)	0.010 (10%)	0.002 (6%)	0.016(14%)
Within person σ^2 (%)[b]	0.021 (66%)	0.011 (18%)	0.018 (31%)	0.057 (84%)	0.089 (90%)	0.043 (96%)	0.100 (86%)
Months apart (*n*=265)							
Spot sample							
ICC	0.21	0.51	0.18	0.01	0.01	0.01	0.01
Between persons σ^2 (%)[a]	0.021 (21%)	0.051 (51%)	0.019 (18%)	0.001 (1%)	0.001 (1%)	0.001 (1%)	0.001 (1%)
Within person, between day σ^2 (%)[b]	0.041 (42%)	0.021 (21%)	0.044 (43%)	0.031 (23%)	0.048 (25%)	0.045 (36%)	0.185 (36%)
Within person, within day σ^2 (%)[c]	0.036 (37%)	0.029 (28%)	0.041 (39%)	0.102 (76%)	0.137 (74%)	0.078 (63%)	0.318 (63%)

First morning sample[d]							
ICC	0.29	0.59	0.31	0.10	0.11	0.09	0.01
Between person σ^2 (%)[a]	0.024 (29%)	0.051 (59%)	0.035 (31%)	0.011 (10%)	0.011 (11%)	0.001 (9%)	0.001 (1%)
Within person σ^2 (%)[b]	0.058 (71%)	0.036 (31%)	0.078 (69%)	0.096 (90%)	0.090 (89%)	0.113 (99%)	0.322 (99%)
24-h collection[d]							
ICC	0.30	0.65	0.30	0.01	0.01	0.02	0.01
Between person σ^2 (%)[a]	0.021 (30%)	0.051 (65%)	0.025 (30%)	0.001 (1%)	0.001 (1%)	0.001 (2%)	0.001 (1%)
Within person σ^2 (%)[b]	0.050 (70%)	0.027 (35%)	0.058 (70%)	0.073 (99%)	0.086 (99%)	0.054 (98%)	0.176 (99%)

Days apart: days 0, 1, 2, 3, and 4; months apart: days 0, 30, 60, and 90. σ^2= variance. [a]The proportion of between-person variance to the total variance. [b]The proportion of within-person between-day variance to the total variance. [c]The proportion of within-person within-day variance to the total variance. [d]For first morning and 24-h urine collections, the distinction between within-day vs. between-day variability is not applicable with only one measurement per day.

Table 4. Creatinine-corrected models of 24-h metal excretions using same-day spot or first morning urine samples as predictors[a].

Type of sample	Mixed regression model			Pearson correlation	
	β (95% CI)	Intercept	Estimated R^{2c}	r[d]	*P*-Value[d]
Spot sample (*n*=88)[b]					
As	0.66 (0.55, 0.76)[**]	2.38	0.67	0.82	<0.001
Cd	0.63 (0.52, 0.75)[**]	1.66	0.76	0.87	<0.001
Co	0.63 (0.52, 0.74)[**]	1.45	0.71	0.84	<0.001
Cu	0.55 (0.47, 0.63)[**]	2.92	0.69	0.83	<0.001
Pb	0.46 (0.31, 0.61)[**]	3.10	0.29	0.54	<0.001
Mo	0.51 (0.39, 0.64)[**]	3.78	0.44	0.66	<0.001
Ni	0.47 (0.36, 0.57)[**]	2.90	0.48	0.69	<0.001
First morning sample (*n*=88)					
As	0.79 (0.70, 0.88)[**]	1.44	0.81	0.90	<0.001
Cd	0.79 (0.70, 0.88)[**]	0.96	0.85	0.92	<0.001
Co	0.69 (0.60, 0.78)[**]	1.26	0.76	0.87	<0.001
Cu	0.70 (0.54, 0.86)[**]	2.02	0.45	0.67	<0.001
Pb	0.55 (0.36, 0.74)[**]	2.71	0.28	0.53	<0.001
Mo	0.50 (0.39, 0.62)[**]	3.91	0.49	0.70	<0.001
Ni	0.57 (0.44, 0.70)[**]	2.35	0.48	0.69	<0.001

[a]Spot and first morning urine samples were analyzed on the $\log_{10}$ scale. [b]Spot urine samples were randomly collected from 11 person on each day. [c]Estimated from single variable linear regression model. [d]Estimated from Pearson's correlation analyses.
[**]*P*-value<0.001.

Table 5. Sensitivity and specificity[a] for classifying participants with high (Top 33%) 3-month average metal concentrations[b] with one, two and three urine samples.

Type of sample collection	Sensitivity (Specificity)						
	As	Cd	Co	Cu	Pb	Mo	Ni
Spot sample							
One sample	0.57 (0.75)	0.77 (0.84)	0.57 (0.80)	0.53 (0.78)	0.50 (0.73)	0.47 (0.71)	0.40 (0.70)
Two samples[c] (days apart)	0.60 (0.80)	0.97 (0.93)	0.60 (0.74)	0.63 (0.80)	0.57 (0.78)	0.50 (0.69)	0.53 (0.75)
Two samples[c] (months apart)	0.63 (0.75)	0.80 (0.81)	0.67 (0.76)	0.67 (0.78)	0.57 (0.75)	0.60 (0.78)	0.43 (0.74)
Three samples[c] (days apart)	0.63 (0.76)	0.97 (0.91)	0.67 (0.78)	0.73 (0.79)	0.70 (0.76)	0.67 (0.79)	0.50 (0.70)
Three samples[c] (months apart)	0.63 (0.73)	0.87 (0.88)	0.80 (0.81)	0.70 (0.78)	0.63 (0.78)	0.63 (0.75)	0.67 (0.78)
k^d	4	3	5	13	20	6	27
First morning sample							
One sample	0.40 (0.69)	0.83 (0.88)	0.57 (0.84)	0.50 (0.73)	0.37 (0.78)	0.47 (0.70)	0.43 (0.74)
Two samples[c] (days apart)	0.63 (0.75)	0.97 (0.95)	0.73 (0.81)	0.67 (0.76)	0.33 (0.71)	0.40 (0.66)	0.67 (0.79)
Two samples[c] (months apart)	0.50 (0.73)	0.97 (0.98)	0.70 (0.81)	0.40 (0.70)	0.43 (0.71)	0.67 (0.78)	0.27 (0.65)
Three samples[c] (days apart)	0.70 (0.78)	0.97 (0.98)	0.70 (0.78)	0.73 (0.80)	0.47 (0.74)	0.60 (0.73)	0.73 (0.80)
Three samples[c] (months apart)	0.53 (0.73)	0.97 (0.98)	0.83 (0.83)	0.30 (0.64)	0.43 (0.68)	0.73 (0.76)	0.20 (0.58)

Days apart: days 0, 1, 2, 3, and 4; months apart: days 0, 30, 60, and 90. [a]Average sensitivity and specificity was computed based on ten data sets each containing one randomly selected urine sample per person. [b]Calculations use creatinine-corrected metal concentrations (μg/g creatinine) on the $\log_{10}$ scale. [c]Urine samples were collected on different days. [d]The minimum number of spot samples (including first morning samples) required to estimate the participant-specific mean within 20% of the "true" value without regard to the day of sample collection.

Figure Legends

Figure 1. Creatinine-corrected metal concentrations (µg/g creatinine) in the urine collected from 11 men on 8 days during a 3-month period. Each graph represents an individual participant (labeled as P1-P11). The dots in each graph represent the metal concentrations in each spot urine sample (including first morning samples) collected on each sampling day.

Figure 2. Scatter plots of correlations between creatinine-corrected metal concentrations (log$_{10}$-transformed) in a 24-h collection and the concentrations measured in a spot sample (randomly selected) or a first morning sample within the same 24-h period (*n*=88). Drawn lines represent the linear trend (all *P*-value <0.001).
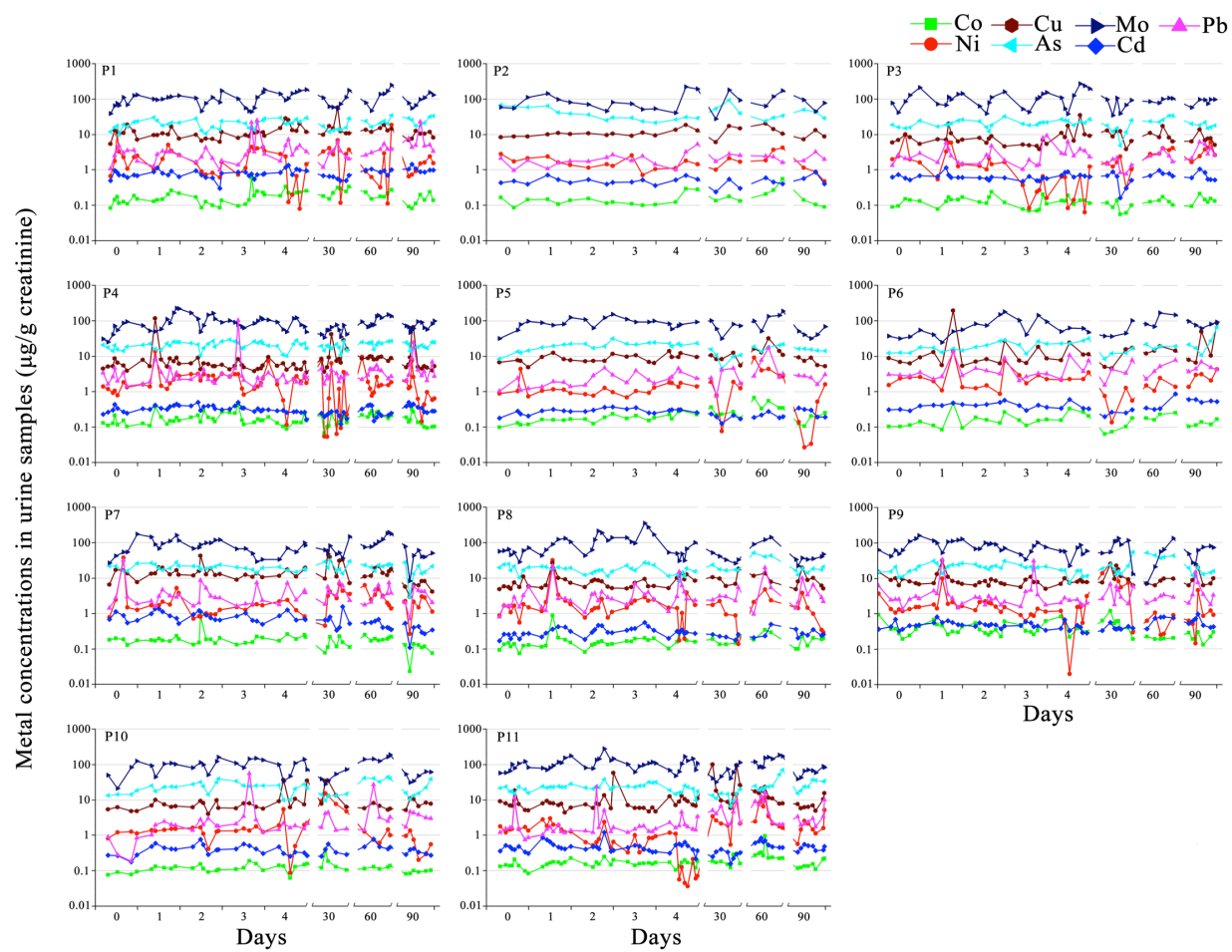
Figure 1.

Figure 2.